Overexpression of *miR-3151* leads to direct deregulation of the TP53 pathway and is associated with *BRAF* mutations in malignant melanoma

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Introduction

The *BRAF* gene is the most frequently mutated gene in malignant melanoma (MM). When mutated, it is associated with a more aggressive disease phenotype. MicroRNAs (miRs) are small non-coding RNAs that downregulate the expression of their target genes by binding to their 3′-UTR.

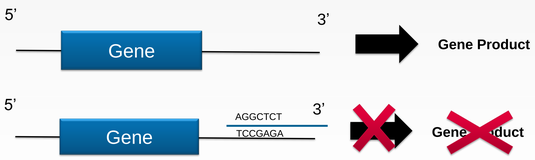


Figure 1: microRNAs bind to the complementary sequence in the 3' UTR of their target gene to downregulate gene expression via various mechanisms.

Recently, microRNA *miR-3151* was identified in intron 1 of *BAALC,* the most upregulated gene in *BRAF* mutated (*BRAF*mut) MM. In acute myeloid leukemia, both high *miR-3151* and high *BAALC* are associated with poor survival. In addition, *miR-3151* has leukemogenic activity via direct deregulation of TP53.

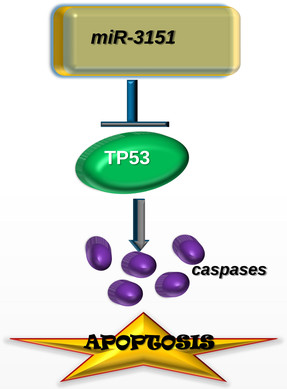


Figure 2: *miR-3151* deregulates TP53. Normally, TP53 activates caspases, which promote programed cell death (apoptosis). *miR-3151* suppresses TP53, leading to decreased caspase activity, and reduced apoptosis.

We hypothesized that *miR-3151* is deregulated in MM and leads to deregulation of the TP53 apoptosis pathway. Furthermore, we hypothesized that *miR-3151* expression may be increased by *BRAF* mutations and may contribute to the increased disease aggressiveness of *BRAF*mut MM.

Methods

Stable *miR-3151* expression: To stably express *miR-3151* in MM cell lines (A375, Mel-39, MeWo), we used the *miR-3151* hairpin sequence with 200 bp flanking sequence cloned into a HIV-based lentiviral expression vector.

Quantifying TP53 expression: To assess the effects of forced *miR-3151* expression on *TP53* expression, RNA was harvested 12h after infection of MM cell lines (A375, MeWo) and *TP53* expression was determined using quantitative PCR (qPCR) to quantify RNA expression.
In addition, protein was harvested 24h after infection and TP53 expression was analyzed using Western blots.

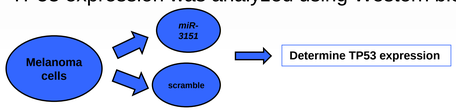


Figure 3: Effects of *miR-3151* on TP53 expression: MM cell lines were infected with the *miR-3151* virus and RNA and protein were harvested to determine TP53 expression.

Gene expression manipulation: siRNA-mediated knockdown was used to silence BRAF in *BRAF*mut MM cell lines (A375, Mel-39). *miR-3151* and *TP53* expression was determined using qPCR.
Furthermore, we transfected the BRAF wild-type (*BRAF*wt) cell line (MeWo) with plasmids containing either the *BRAF*wt or *BRAF*mut vectors.

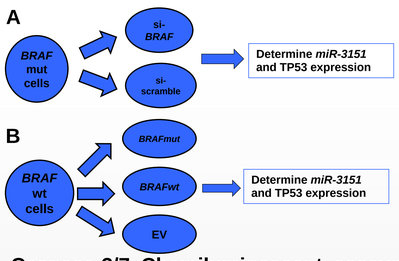


Figure 4: (A) We silenced BRAF in *BRAF*mut MM cell lines and then determined *miR-3151* and *TP53* expression. (B) We overexpressed BRAF wild-type and introduced the *BRAF* mutation in *BRAF*wt MM cell line.

Caspase-3/7 Chemiluminescent assays: We performed caspase-3/7GLO assays with *miR-3151* or antago*miR-3151* infected MM cell lines (A375, Mel-39) to see a change in apoptosis rates caused by expression manipulation of *miR-3151.*

Flow cytometry: Flow cytometry was used to distinguish which phase cells were undergoing in the cell cycle (G1, S, G2) in an antago*miR-3151* infected MM cell line (Mel-39) compared to scramble control.

Results

miR-3151 targets *TP53* in MM cell lines

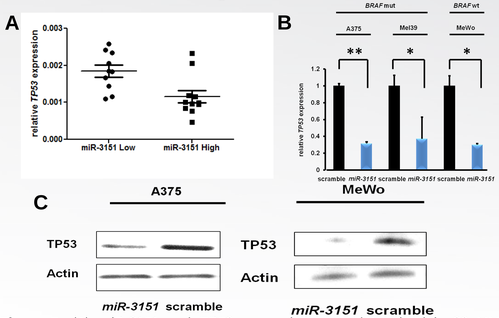


Figure 5: (A) *miR-3151* and *TP53* expression were determined in 20 MM patients. Patients with high *miR-3151* expression had lower *TP53* expression. *miR-3151* was overexpressed in MM cell lines. Forced expression of *miR-3151* lead to decreased TP53 expression as seen by (B) qPCR and (C) Western blots.

miR-3151 decreases apoptosis in MM cell lines

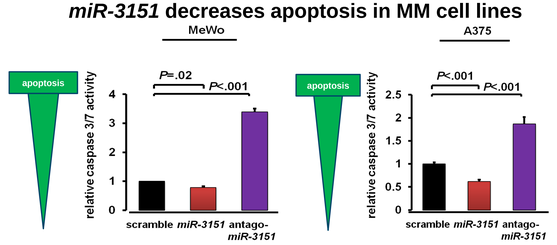


Figure 6: Caspase-3/7 activity was measured in *miR-3151* and antago*miR-3151* infected MM cell lines (MeWo, A375) compared to scramble control. *miR-3151* decreased caspase activity, while antago*miR-3151* increased caspase activity.

antago*miR-3151* reduces cell proliferation in melanoma cell lines

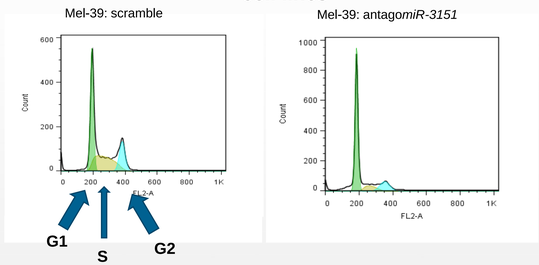
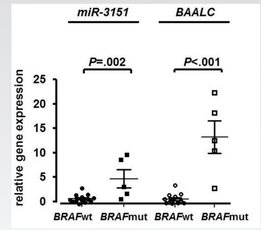


Figure 7: The effect of antago*miR-3151* on the cell cycle in Mel-39 cells was examined by flow cytometry. The number of cells in S and G2 phases were reduced in the antago*miR-3151* infected cells compared to scramble control, suggesting that antog*miR-3151* reduces cell proliferation of MM cells.

*BRAF*mut patients have higher *miR-3151* expression

Figure 8: *miR-3151* and *BAALC* expression levels were determined in 20 MM patient samples. The *BRAF* mutation status was then determined. *BRAF*mut patients had a 5-fold higher *miR-3151* expression compared to *BRAF*wt patients.



BRAF mutations can directly influence *miR-3151* expression

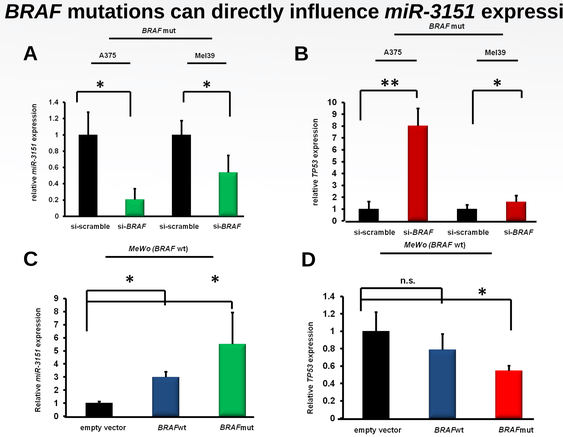


Figure 9: (A) BRAF was silenced in *BRAF*mut MM cell lines (A375, Mel-39) and *miR-3151* expression was determined compared to si-scramble control. (B) *TP53* expression was measured in the *BRAF*mut cell lines after silencing BRAF compared to scramble control. (C) *BRAF*wt was overexpressed and the *BRAF* mutation was introduced in a *BRAF*wt cell line (MeWo), then *miR-3151* expression and (D) *TP53* expression were determined compared to empty vector.

Future directions

Next steps: 1) To see if other *BRAF* mutated malignancies (papillary thyroid cancer) have increased *miR-3151* expression.
2) To see if the efficacy of Vemurafenib is increased when used in conjunction with the antago*miR-3151* virus in MM.

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